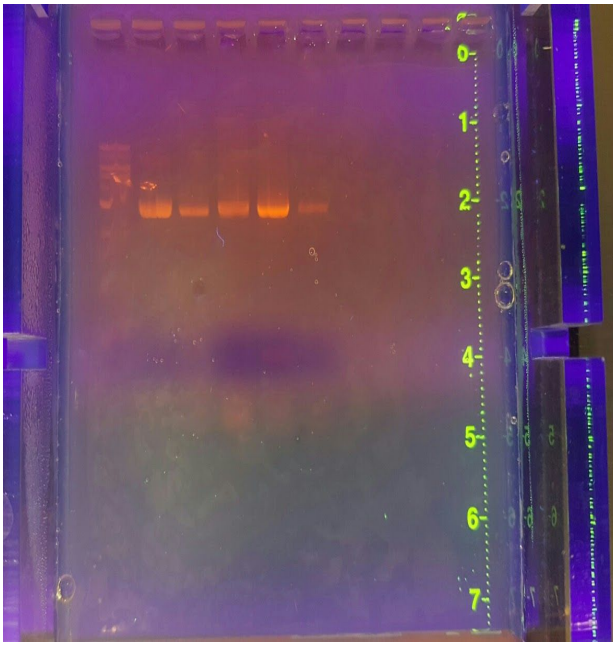
iGEM 2016: Pittsburgh
Week 11 Lab Notebook

Monday, August 1

PCR of RBS-T3 plasmid using different primers and temperatures (Maddie/ Maya)
- Xba-T3 Reverse 1 and EcoRI-T3 Forward 1 (52℃)
- Xba-T3 Reverse 1 and EcoRI-T3 Forward 1 (55℃)
- PST-T3 Reverse 2 and Xba T3-Forward 2 (52℃)
- PST-T3 Reverse 2 and Xba T3-Forward 2 (55℃)

Gel of RBS-T3 PCR (Maddie/ Maya)
- Lane 1: 1 kb ladder
- Lane 2: Control (uncut RBS-T3)
- Lane 3: Xba-T3 Reverse 1 and EcoRI-T3 Forward 1 (52℃)
- Lane 4: Xba-T3 Reverse 1 and EcoRI-T3 Forward 1 (55℃)
- Lane 5: PST-T3 Reverse 2 and Xba T3-Forward 2 (52℃)
- Lane 6: PST-T3 Reverse 2 and Xba T3-Forward 2 (55℃)



Tuesday, August 2

Amplification of T3 from Lane 5 on previous gel (Maddie/ Maya)

Anneal DNAzyme at higher ratios, D and G (Claire and Praneeth)

1:5	1 uL 2.5 uM substrate
50 nM : 250 nM	5 uL 2.5 uM catalytic
	5 uL 10X T4 ligase buffer
	39 uL H2O
1:10	1 uL 2.5 uM substrate
50 nM : 250 nM	10 uL 2.5 uM catalytic

	5 uL 10X T4 ligase buffer
	34 uL H2O
1:25	1 uL 2.5 uM substrate
50 nM : 250 nM	5 uL 2.5 uM catalytic
	25 uL 10X T4 ligase buffer
	19 uL H2O
1:50	1 uL 2.5 uM substrate
50 nM : 250 nM	25 uL 5 uM catalytic
	5 uL 10X T4 ligase buffer
	19 uL H2O
1:100	1 uL 2.5 uM substrate
50 nM : 250 nM	25 uL 10 uM catalytic
	5 uL 10X T4 ligase buffer
	19 uL H2O

Wednesday, August 3

Colony PCR of 3:1 PT3-RBS-Backbone, 7:1 PT3-Backbone, 7:1 PT3-RBS-Backbone (Maddie/ Maya)

2.5% gel of colony PCR (Maddie)

- Lane 1: 1kb ladder
- Lane 2: control (gfp plasmid)
- Lane 3: 3:1 PT3-RBS-Backbone
- Lane 4: 3:1 PT3-RBS-Backbone
- Lane 5: 7:1 PT3-Backbone
- Lane 6: 7:1 PT3-Backbone
- Lane 7: 7:1 PT3-Backbone
- Lane 8: 7:1 PT3-RBS-Backbone
- Lane 9: 7:1 PT3-RBS-Backbone
- Lane 10: 7:1 PT3-RBS-Backbone
- Maya Insert Gel Pic

Anneal DNAzyme at higher ratios, D and G (Claire)

1:200	1 uL 2.5 uM substrate
50 nM : 10 mM	10 uL 50 uM catalytic
	5 uL 10X T4 ligase buffer
	34 uL H2O
1:500	1 uL 2.5 uM substrate
50 nM : 25 mM	25 uL 50 uM catalytic
	5 uL 10X T4 ligase buffer
	19 uL H2O

InterLab Study: OD_{600} Reference Point (Claire)

Replicate	1	2	3	4
LUDOX	0.049	0.051	0.050	0.051

Water 0.045 0.045 0.046 0.045

InterLab Study: FITC Standard Curve (Claire)

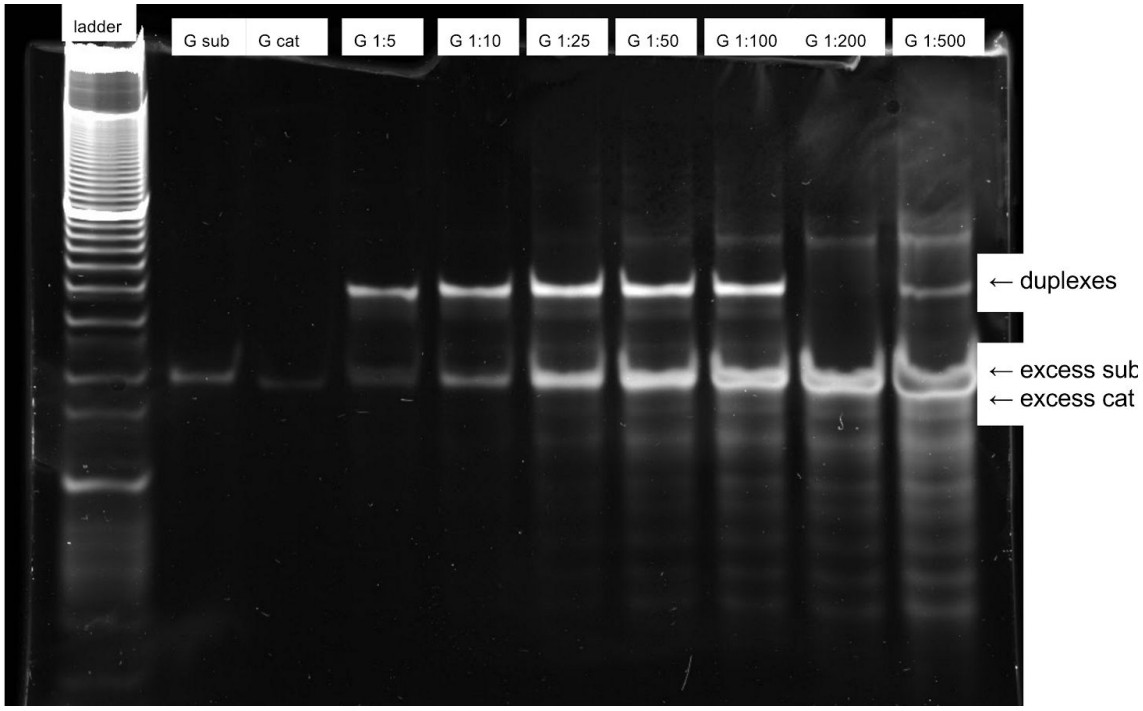
	250.00	125	62.5	31.25	15.625	7.8125	3.90625	1.95312	0.97656	0.48828	0.24414	0
1	14026.7	8560.987	5160.085	3035.062	1749.963	1045.47	668.82	438.777	329.76	275.73	232.645	177.644
2	17027.8	6688.731	6287.875	3140.935	1886.993	1101.504	707.469	466.514	324.182	271.022	229.897	174.433
3	16755.0	4215.326	8489.458	3497.327	2015.3	1210.591	757.509	499.559	347.338	283.881	236.375	179.959
4	14274.4	8426.716	5253.528	3252.416	1649.179	1024.914	707.624	402.89	345.437	261.159	247.028	175.793

Fluorescence emission 485, excitation 515, cutoff 495

96 well clrbtm

PAGE DNAzyme annealing check (Claire and Praneeth)

Samples: 9 uL plus 9 uL loading buffer



Hard to differentiate substrate and catalytic strands

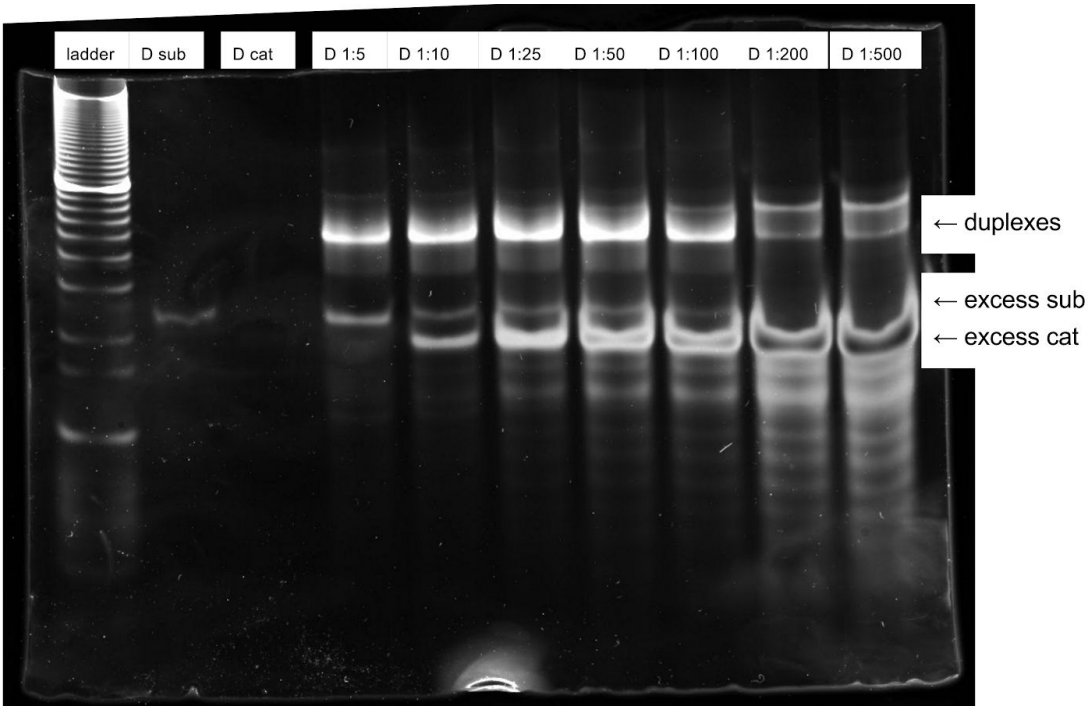
Not sure why duplexes become fainter with 1:200 and 1:500

Annealed by Claire that morning?

Looks like excess of catalytic starting from 1:10?

Hard to tell--run gel again tomorrow, perhaps include 1:500

Decrease amount of DNA loaded as ratio increases



Not sure why catalytic strand control disappeared again
Catalytic strand is larger than substrate strand; probably the band underneath it
1:10 starts producing excess of catalytic, but still excess substrate until 1:200
Re-run gel up to 1:200, load less DNA in the higher ratios (starting from 1:25 ish)

Meeting with Dr. Daniel Bain (Claire, Maya, Aife, Praneeth)

Oxidation states: Tl^{+1} halides stable
Alkali earth (not very soluble)
Tl^{+3} reduced with any reductant
Unstable

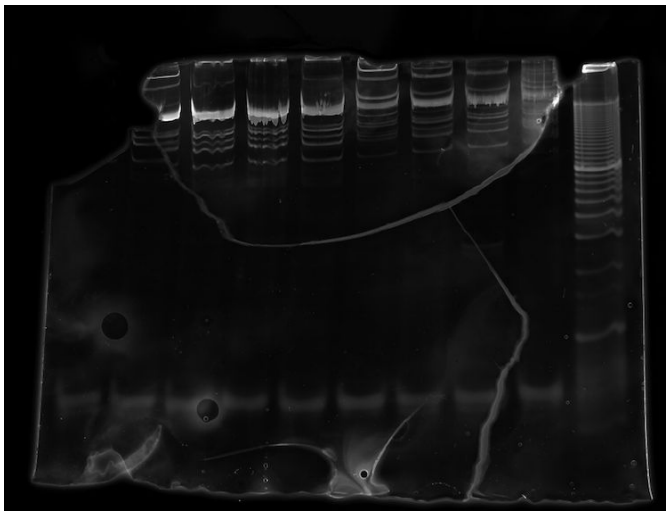
Solubility--how much is actually in water? Ksp = 2×10^{-44}
Thallium won't necessarily be in solution
Like Cr, with which Dr. Bain works

Current detection: ICPMS
Market for cheap lead screening tool
Current lead test: 10 to 25 dollars (especially because of paperwork)
Pre-screen for agencies (quality control)
Also for arsenic, antimony, barium (EPA MCL's)
Drinking water is constant matrix (the other things that are also dissolved)
Practicality considerations:
Tl solubility in water
Threshold of detection (MCL is 50 parts per trillion)

Thursday, August 4

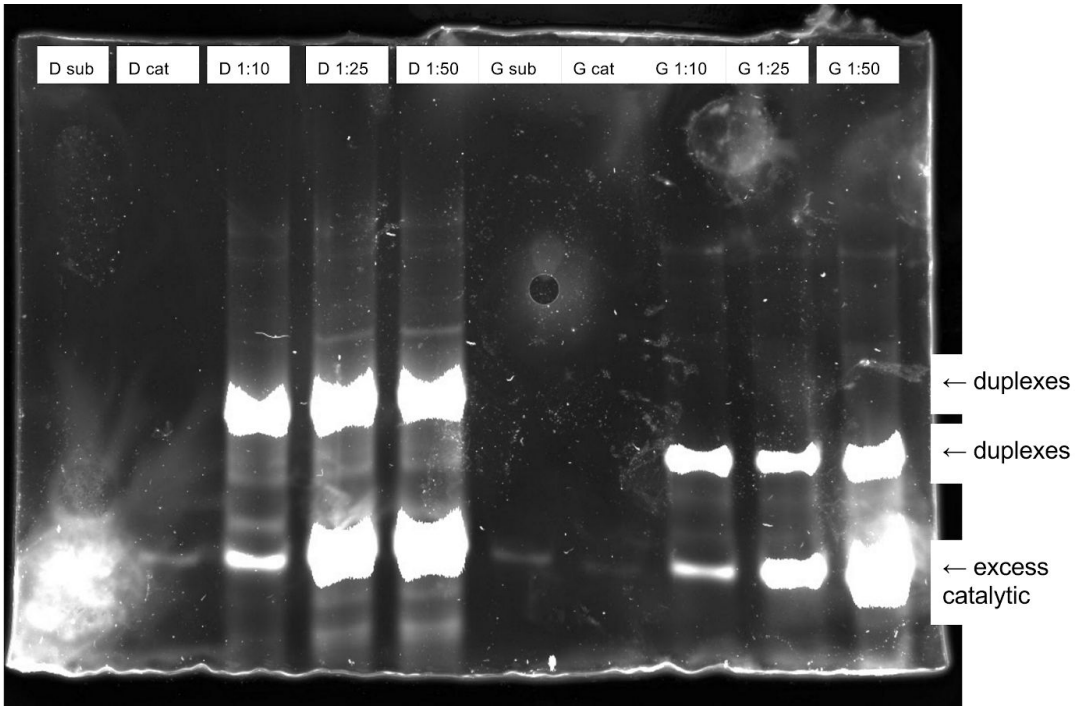
Camp BioE presentation (Claire and Praneeth)
Colony PCR with acrylamide gel (Maddie/Maya)

Made Colony PCR Master Mix for 10 rxns (9 used)
Used BioBrick Primer setting on PCR machine
(reversed)
Lane 10: 7:1 PT3-RBS-Backbone
Lane 9: 7:1 PT3-RBS-Backbone
Lane 8: 7:1 PT3-Backbone
Lane 7: 7:1 PT3-Backbone
Lane 6: 7:1 PT3-Backbone
Lane 5: PT3-RBS-Backbone
Lane 4: PT3-RBS-Backbone
Lane 3: Negative control (backbone)
Lane 2: Positive control (GFP plasmid)
Lane 1: 10 bp ladder



Liquid cultures of colonies (Maddie)
DNAzyme duplexes on native gel (Claire and Praneeth)
12% gel for better separation between catalytic and substrate strands
3 mL 40% acrylamide
7 m 1X TBE buffer
100 uL 10% APS
15 uL TEMED

Samples:	substrate, catalyst, 1:10	9 uL plus 9 uL buffer
	1:25, 1:50	8 uL plus 8 uL buffer



1:10 produces excess of catalytic for both

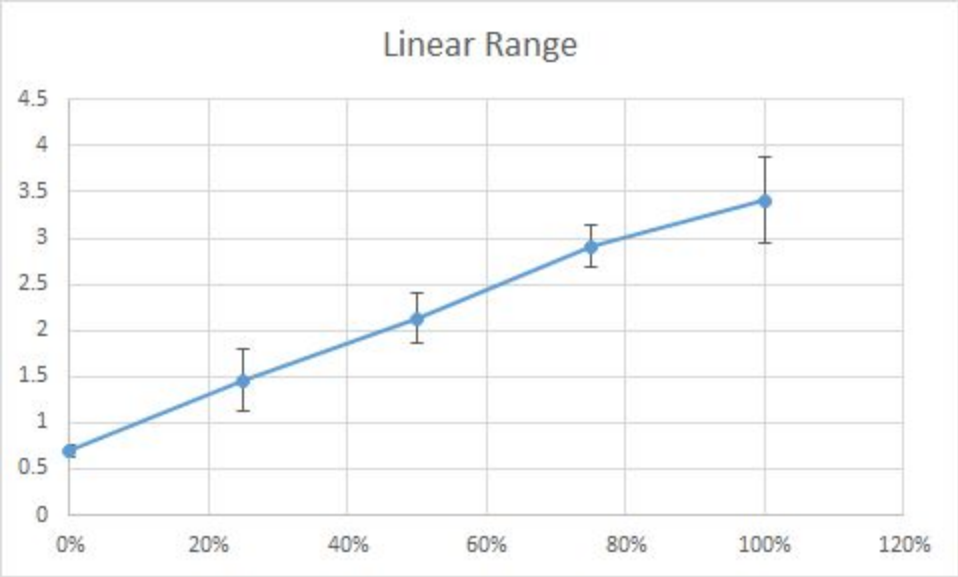
D substrate strand not completely sequestered even in excess of catalytic

Collaboration: [UGA Archaeal InterLab] (Claire)

Linear range

Sample C triplicate readings, 50 uL total volume, dilutions with water 100%, 75%, 50%, 25%, 0%

	replicate 1	replicate 2	replicate 3	average	st dev
0%	0.623	0.748	0.727	0.699333	0.066935
25%	1.127	1.464	1.791	1.460667	0.332013
50%	2.362	2.185	1.836	2.127667	0.267646
75%	2.736	3.162	2.839	2.912333	0.222266
100%	2.94	3.869	3.406	3.405	0.464501



50 uL volume, no dilution, is within range

Reading, 50 uL of each sample per well

Excitation: 590 nm Emission: 645 nm Cutoff: 630 nm

	replicate 1	replicate 2	replicate 3	average	st dev
A	0.626	0.634	0.716	0.658667	0.049813
B	0.363	0.398	0.255	0.338667	0.074541
C	3.25	3.322	2.704	3.092	0.337941
WT	0.62	0.528	0.269	0.472333	0.182001
S	0.577	0.377	0.387	0.447	0.112694

Anneal DNAzyme strands at higher concentration for cleavage reaction (Claire)

D and G, 8 uM : 80 uM

2 uL 100 uM substrate strand

23.5 uL 85 uM catalytic strand

2.5 uL T4 ligase buffer

DNAzyme cleavage reaction

0.7 uM = 1.4 uL annealed oligos

10 uM = 0.5 uL 1 mM erbium

23.35 uL buffer B

Mixed 2x buffer B and 2x erbium together, then separated into tubes for D/G

Forgot to quench; stored in freezer about 2 hours after incubation period

Quick cell-free cleavage check

Single replicates

25 ng of switch to “save room” for trigger

Switch G	control	duplex	Completed cleavage	Cleavage components	DNA trigger
Solution A	2	2	2	2	2
Solution B	1.5	1.5	1.5	1.5	1.5
RNAse inhibitor	.25	.25	.25	.25	.25
substrate	.25	.25	.25	.25	.25
Switch (25 ng)	.30	.30	.30	.30	.30
“trigger”	0.7 water	0.7 50 nM : 500 nM	.7 completed rxn	.28 8 uM duplex, .25 1 mM Er, .17 B	0.7 uL at 2.5 uM
ABSORB@570	0.1	0.1121	0.2683	0.2565	1.2161

Control was not part of master mix; ran out

Switch D	Duplex	Completed reaction
Solution A	2	2
Solution B	1.5	1.5
RNAse inhibitor	.25	.25
substrate	.25	.25
Switch (25 ng)	.33	.33
“trigger”	0.67 8 uM : 80 uM	.67 completed rxn
ABSORBANCE @570 nm	0.0615	0.0604

Couldn’t find 50 nM : 500 nM D duplex

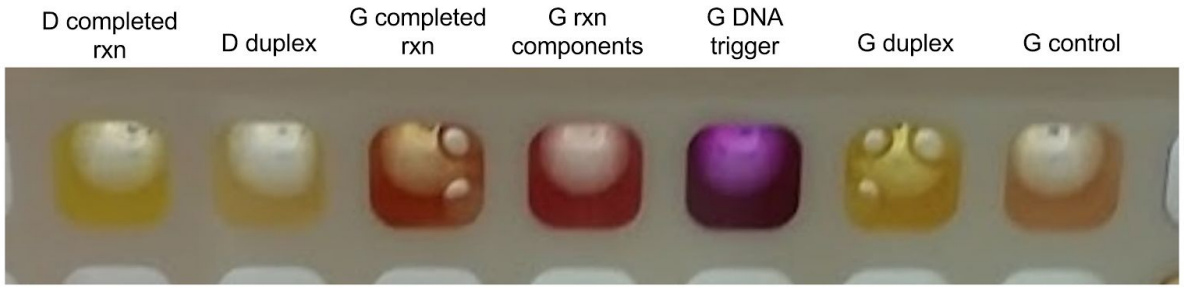
Master mix: 14 uL Solution A
10.5 uL Solution B
1.75 uL RNAse inhibitor
1.75 uL substrate

Remove 8 uL for D, add 0.66 uL D switch
Separate into 2 x 4.33 uL, add appropriate triggers

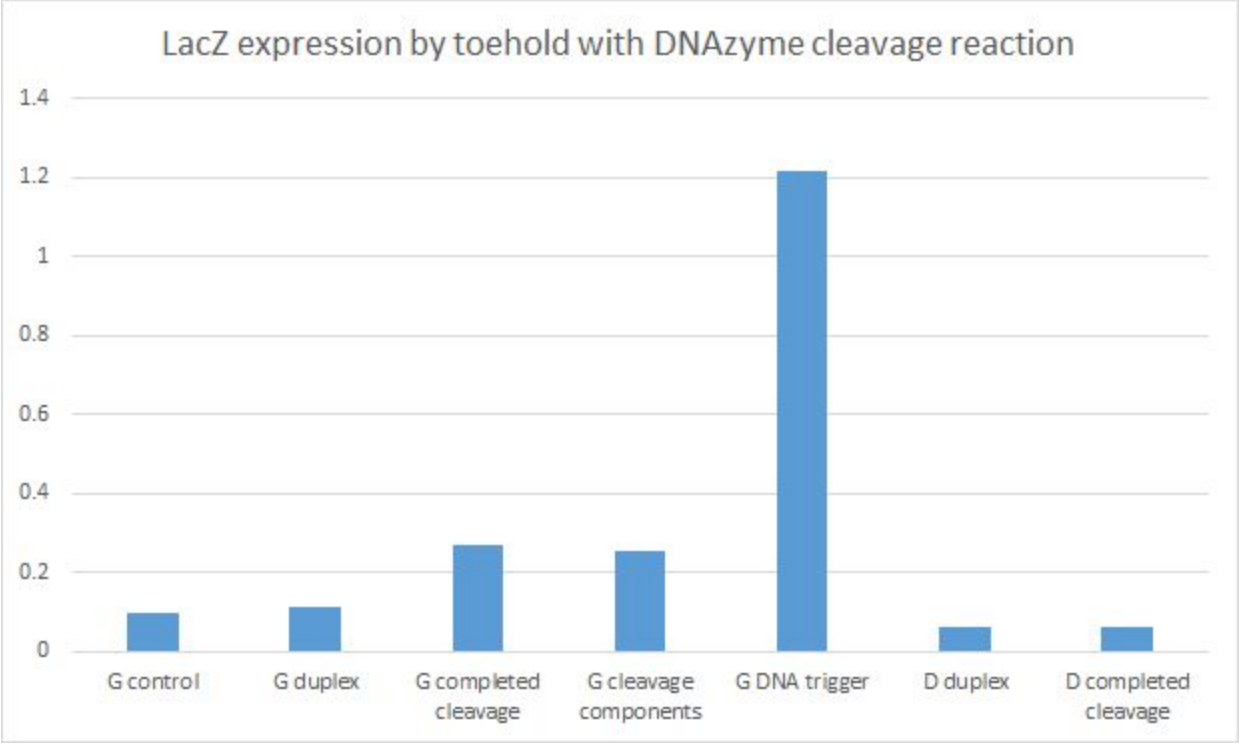
Add 1.5 uL G switch
Separate into 4 x 4.3 uL, add triggers

Pipette G control separately (ran out of control)

Dilute to 10 uL total volume to read in 384-well plate
Neither D duplex nor cleavage reaction activates toehold
G control and duplex produce similar minimal toehold activation
G reaction and completed reaction produce similar medium activation
*D duplex should even be more concentrated than G duplex



	Absorbance
G control	0.1
G duplex	0.1121
G completed cleavage	0.2683
G cleavage components	0.2565
G DNA trigger	1.2161
D duplex	0.0615
D completed cleavage	0.0604

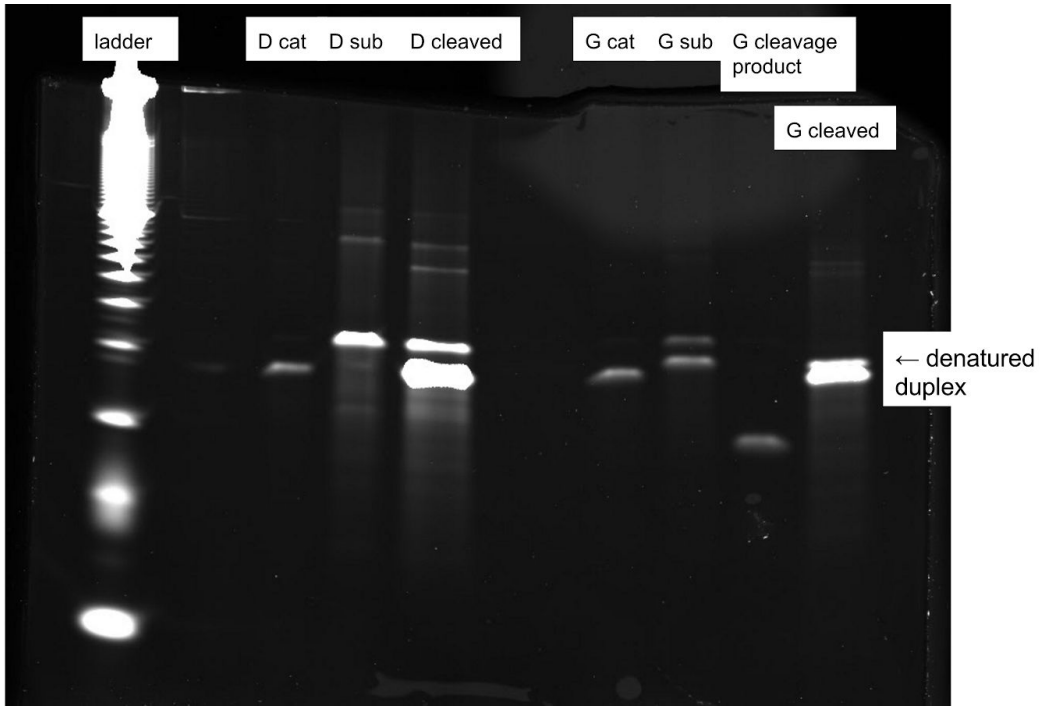


Friday, August 5

dPAGE of cleavage reactions (Claire)

Lane 10 1 uL ladder + 1 uL loading buffer
8 10 uL 50 nM D catalytic + 10 uL buffer
7 10 uL 50 nM D substrate + 10 uL buffer
6 2 uL D cleavage reaction (assume 0.7 uM) + 2 uL buffer
4 10 uL 50 nM G catalytic + 10 uL buffer
3 10 uL 50 nM G substrate + 10 uL buffer
2 10 uL 50 nM G cleavage product + 10 uL buffer
1 2 uL G cleavage reaction (assume 0.7 uM) + 2 uL buffer

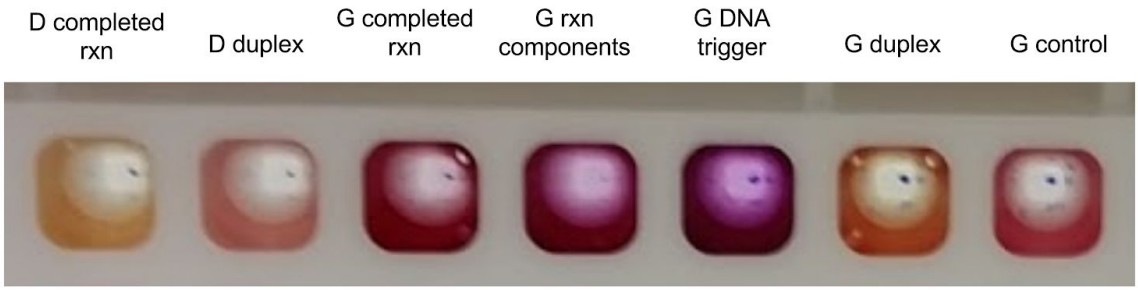
Steve's door locked; couldn't get D DNA oligo trigger
Heat all samples except ladder at 95 degC for 5 min
Spin down after heating to collect evaporated liquid
Heat 8-6 while loading ladder
Heat 7-10 while loading 8-6
Run at 100 V for 1 hour
No loading buffer blots--ran other way? Gel inserted front-to-back
Same gel, turned the right way. Loaded same samples in each lane.
Run at 100V for 15 min, 150 V for (looked slow)
Samples don't sink to bottom of well; remain suspended wherever they're left



Substrate strands have a second band for some reason
No cleavage product visible--efficiency probably too low

Cell-free quick test overnight incubation (Claire)

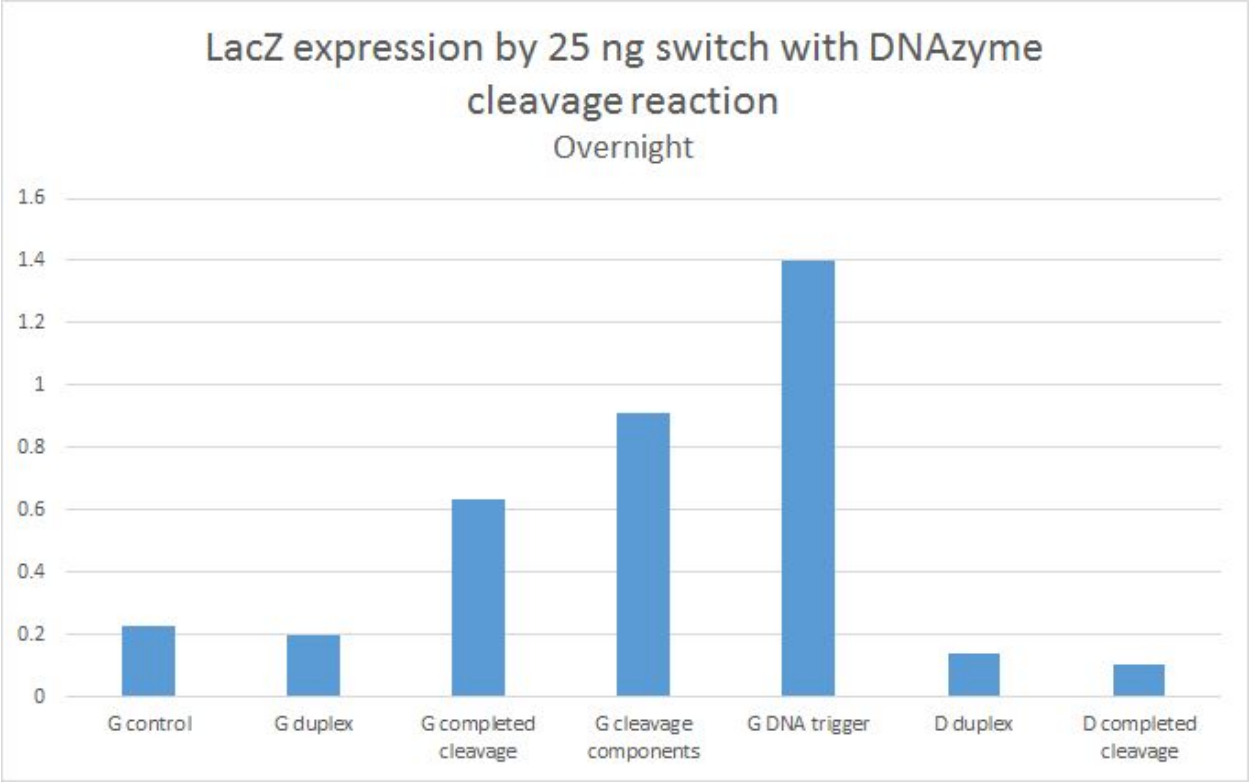
Rehydrate wells with 10 uL water



D duplex confirms that not all substrate strand is sequestered

Why is G control so purple? What does that mean for G duplex's activation?

	Absorbance
G control	0.2289
G duplex	0.1958
G completed cleavage	0.6361
G cleavage components	0.9087
G DNA trigger	1.3989
D duplex	0.1367
D completed cleavage	0.1038



Sent out 10 reactions to Genewiz for sequencing (Maya)

Mixture of PT3 and PT3-RBS